OVULATORY FOLLICULAR DEVELOPMENTS AND PLASMA PROGESTERONE PROFILES IN SUPEROVULATED DAIRY COWS

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ABSTRACT

A study concerning follicular development and plasma progesterone concentrations had been conducted in 9 heads of superovulated dairy cows. Superovulatory treatments consisted of treatment I with multiple injection of FSH 6 - 6, 5 - 5, 4 - 4 and 3 - 3 AU with solvent of aquabidest; treatment II with single injection of 30 AU FSH with solvent of PVP; and treatment II with single injection of 3,000 IU PMSG followed by 5,000 IU HCG at the time of the first insemination. Embryo recovery and evaluation were done at the day 7 or 8 after insemination.

Ovulatory follicular development was monitored using transrectal real-time ultrasonography. Plasma progesterone determination was performed using a microtitre plate enzyme immunoassay technique. Ultrasonographic examination and blood sample collection were done every other day commencing the day of superovulatory treatment until the time of embryo recovery.

Results of the present study showed that superovulatory treatments I and II had the same number of ovulatory follicular development (12.5 ± 0.5 and 15.5 ± 2.5, $P > 0.05$), while treatment III had fewer follicular development (11.0 ± 1.0, $P < 0.05$). Plasma progesterone levels in treatments I and II at the time of embryo collection were also the same, 9.4 ± 3.1 dan 8.8 ± 2.4 ng/ml ($P > 0.05$), respectively; while in treatment III had lower concentration 4.2 ± 1.3 ng/ml ($P < 0.05$). The higher incidence of anovulatory follicle and lower embryo quality in treatment III seemed due to this lower plasma progesterone level.

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In conclusion, superovulatory treatment using FSH reveals better results compared with the use of PMSG. The better results are manifested with more ovulatory developments and rates, as well as higher plasma progesterone concentrations. Single injestion of FSH with PVP solvent gives no differences in superovulatory response and in plasma progesterone level with multiple injection with aquabidest solvent.

Key words : embryo transfer, superovulation, follicular development, ovulatory follicle, plasma progesterone.

INTRODUCTION

Superovulation is the main technique to increase the efficiency of genetic superior donor utilization in embryo transfer application. The standard technique that is widely utilized is the administration of *pregnant mare's serum gonadotrophin* (PMSG) 3,000 IU combined with *human chorionic gonadotrophin* (HCG) 5,000 IU or with injection of *follicle-stimulating hormone* (FSH) 8 times, multiple dosages with interval 12 hours between each injection (Suzuki, 1991, 1993). PMSG has adverse effects to the superovulatory response since its high anatigenicity character, as well as overstimulatory effects to the ovaries (Kamomae, 1990). The use of FSH with multiple injections, beside less practical also causes unnecessary stress to the donor animals. A simpler and more advantageous superovulatory treatment, using FSH with solvent of polyvinyl pyrrolidone (PVP, molecular weight = 40,000) given with single injection, had been introduced recently with no signifant differences compared with the conventional methods (Suzuki, 1993).

Superovulatory treatment using FSH followed by administration of prostaglandin will commence multiple follicular developments and simultaneous ovulations, accompanied with the sudden decrease of plasma progesterone levels. The present study was designed to monitor ovulatory follicular developments and plasma progesterone profiles in donor cows following superovulatory treatments using multiple dose of FSH with aquabidest solvent, single dose of FSH with PVP solvent, and also treatment using PMSG combined with HCG.

MATERIALS AND METHODS

Experimental Animals

In the present study 9 heads of parous Friesian Holstein cows, between 5 and/ 8 years old, healthy, non-pregnant, and body scoring according to the method of Gaines (1989) between 3 and 4, were used as experimental animals. The animals had no history of reproductive problems before experiment and had normal estrous cycles in the last 3 cycles, and also had necessary requirements as donor animals as described by Shimohira (1991). The animals were divided into 3 treatment groups, with 3 heads of cow in each group.

Superovulatory Treatment

Superovulatory treatments were performed with intramuscular injection of purified *follicle stimulating hormone* (FSH-W, Antrin^{TM}, Denka Pharmaceutical Co., Japan). For treatment group I, each animal was injected with a total dose of 36 AU FSH in aquabidest, divided into 8 times injections with interval 12 hours, morning and afternoon (6 - 6, 5 - 5, 4 - 4, 3 - 3 AU). For treatment group II, each donor was injected in single dose with 30 AU FSH in 10 ml PVP (Polyvinyl pyrrolidone). In treatment group III, the animal was injected with PMSG (Folligon, Intervet B. V., Boxmeer, Holland) 3,000 IU followed with injection of HCG (Chorulon, Intervet B. V., Boxmeer, Holland) 5,000 IU. The injections of FSH or PMSG were commenced on days 10-12 of the cycle (day of estrus = day 0). All treatment groups were also injected with prostaglandin (Prosolvin, Intervet, B. V., Boxmeer, Holland) 15 mg two times, morning and afternoon, 2 days after the commencement of FSH or PMSG injections. Artificial insemination was performed about 8 hours after the animal showing standing estrus; it was done 3 times with interval 12 hours. Embryo collection and evaluation were performed according to the method of Suzuki (1991).

Ovarian Ultrasonographic Examination

A real time ultrasonographic machine *(real-time ultrasonic scanning instrument)* (Aloka SSD-220, Aloka Co., Matsushita Electric Co., Ltd., Japan) equipped with 5 MHz transrectal transducer, was used for ovarian examinations. The trransducer was wrapped with long plastic sleeve and special lubricant *(scanning gel)* was used to help the ovarian examinations.

Each ovary was examined separately with scanning on its surface from lateral to medial, then from medial to lateral parts, along the longitudinal axis of the ovary. Antra follicle more than 10 mm in diameter were measured with integral electronic caliper at the edge of follicular wall and fluid. Follicle with diameter less than 10 mm was estimated its size by comparing with millimeter scale on the frame of the ultrasound monitor.

Ovarian examination was performed every second day started at the time of superovulatory treatment until several days after the ovulation time of the developing follicles. The day of ovulation was noted by abrupt disappearing of follicles with antra diameters more than 10 mm that seen developing in the ovary several days before. Development of follicle was followed only on follicles having diameter 10 mm or more according to the method of ovarian ultrasonographic examination established by Quirk dan Fortune (1990).

Plasma Progesterone Concentration Assessment

Blood samples were collected every other day soon after ultrasonographic examination until the time of embryo recovery. Blood was drawn from coccygeal vein using vacuum, steril, 10 ml tube, contains 143 USP unit lithium heparin (Vacutainer^{TM}, Beckton Dickinson Co., Rutherford, NJ). Blood samples were then centrifuged at velocity of 2000 *g*, temperature 5°C, for 20 minutes. Blood plasma were then poured into plastic tubes and stored at temperature -20°C until assayed for progesterone contents.

Plasma progesterone determination was performed by the use of a microtiterplate enzyme immunoassay based on the techique developed by Munro dan Stabenfeldt (1989). Sensitivity of this progesterone enzyme immunoassay was 2.2 pg/ml, and cross reactivity to other steroid hormones was < 1%. Intra-asay coefficient was 8.85%, inter-assay coefficient was 11,58%, non-specific binding was 7,2% and extraction recovery rate was 86,5% (Putro. 1991).

Data Analysis

Analysis of regression was done to determine ovulatory follicular development rates, while plasma profesterone concentration pofiles after treatment was anlayzed using *least square of variance* (mean ± SEM). Differences between means of ovulatory follicular developments, plasma progesterone profiles, number of transferable embryos and other parameters were tested using analysis of variance.

RESULTS AND DISCUSSION

Results of superovulatory follicular development, including number of ovulatory follicles, number of ovulation, anovulatory follicles, total embryos and transferable embryos are presented in Table 1. Changes of plasma progesterone profiles commencing from the time of superovulatory treatment until embryo recovery are presented in Table 2.

Table 1. Ovulatory follicular development and number of embryos.

Number	Treatment I Multiple FSH	Treatment II Single FSH	Treatment III PMSG + HCG
Follicle	12.5 ± 0.5^{a}	15.5 ± 2.5^{a}	11.0 ± 1.0^{b}
Ovulation	11.0 ± 1.0^{c}	14.0 ± 3.0^{c}	7.0 ± 1.0^{d}
Anovulation	1.5 ± 0.5^{e}	1.5 ± 0.5^{e}	4.0 ± 0.0^{f}
Total embryos	9.5 ± 1.5^{g}	12.5 ± 2.5^{g}	6.0 ± 1.0^{h}
Transferable embryos	7.0 ± 1.0^{i}	11.0 ± 2.0^{j}	3.5 ± 0.5^{k}

^{a,b,c,d,e,f,g,h,i,j,k} Different superscripts within one row differ significantly ($P < 0.05$).

Table 2. Plasma progesterone profiles in superovulated cows.

Day of Treatment	Treatment I Multiple FSH (ng/ml)	Treatment II Single FSH (ng/ml)	Treatment III PMSG + HCG (ng/ml)
1	8.4 ± 3.2^{a}	7.8 ± 2.8^{a}	7.3 ± 1.8^{a}
3	8.9 ± 4.1^{b}	8.3 ± 3.1^{b}	8.1 ± 2.1^{b}
5 (estrus)	0.4 ± 0.1^{c}	0.6 ± 0.1^{c}	0.5 ± 0.2^{c}
7	1.2 ± 0.2^{d}	1.6 ± 0.3d	0.9 ± 0.1e
9	4.4 ± 1.3^{f}	5.2 ± 2.1^{f}	2.1 ± 0.7^{g}
11	7.3 ± 2.8^{h}	6.7 ± 1.9h	3.8 ± 0.8i
13 (embryo recovery)	9.4 ± 3.1^{j}	8.8 ± 2.4^{j}	4.2 ± 1.3^{k}

a.b.c.d.e.f.g.h.i.j.k Different superscripts within one row differ significantly ($P < 0.05$).

Results of ovulatory follicular developments and ovulation rates of treatment I (multiple injection of FSH) and treatment II (single injection of FSH) were not significantly different ($P > 0.05$). Treatment III (PMSG + HCG) resulted in less ovulatory follicular developments and fewer ovulation rates, as well as higher incidence of anovulations and delayed ovulations ($P < 0.05$). Results of PMSG application in the current study supported the findings of Kamomae (1990), also Mapletoft and Pierson (1983) that observed the signs

of ovarian overstimulation, high cases of delayed ovulations and anovulations, although high doses of HCG (5,000 IU) had been given to overcome the appearing problems.

Ovulatory follicular developments and ovulation rates in treatments I and II did not show any significant differences ($P < 0.05$). The fact proved that the use of PVP for FSH solvent with single injection could substitute the use of aquabidest with multiple injection and resulted in the same superovulatory responses. Suzuki (1993) reported the use of PVP also did not find any differences in its superovulatory responses. The patterns of ovulatory follicular development in the present study were in accordance with the findings of Rajamahendran dan Cadler (1993) that stated there were progressive developments of dominant follicles soon after superovulatory treatments.

In general, in all superovulated animals, plasma progesterone profiles were not different at the time of superovulatory treatment commencement (days 10-12 of the cycle). Then, the progesterone concentrations decreased rapidly following prostaglandin treatment, and reached their minimum levels at the time of estrus. Afterwards, the progesterone levels increased steadily soon after the ovulation time. At the embryo collection time (day 7 or 8), plasma progesterone levels in treatments I dan II were high enough, although they were not significantly different ($P > 0.05$); but in treatment III showed lower levels ($P < 0.05$). Some differences, lower levels of plasma progesterone in PMSG treatment group seemed closely associated with its characteristic of ovarian overstimulatory of this hormone preparation and several cases of anovulatory follicles. The lower progesterone concentrations during this period also resulted in lower quality of eembryos compared with embryos obtained from treatments I and II. The results of the current study were also in agreement with the reports of Anderson (1990) and Assey *et al.* (1993) concerning ovulatory follicles, and Suzuki (1993) dealing with plasma progesterone concentrations and embryo qualities. They noted that for optimum embryo development it was necessary progressive increases of progesterone levels after ovulation and formation of well-established corpora lutea.

Superovulatory treatment using multiple dose of FSH (with aquabidest solvent) and single dose of FSH (with PVP solvent) showing no differences in plasma progesterone profiles. This fact proved that PVP had the ability to solve FSH and then to release FSH slowly but surely. The same finding was also recently reported by Suzuki (1993) in using PVP as FSH solvent for superovulatory treatment in *Japanese Black Cattle* and Limousine.

In conclusion, superovulatory treatment using FSH reveals better results compared with the use of PMSG. The better results are manifested with more ovulatory follicular developments and rates, as well as higher plasma progesterone profiles. Single injection of FSH with PVP solvent gives no differences in the superovulatory responses and in the plasma progesterone profiles with multiple injection of FSH with aquabidest solvent.

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REFERENCES

Assey RJ, Purwantara B, Greve T, Hytell P dan Schmidt MH. (19913). Corpus luteum size and plasma progesterone levels in cattle after cloprostenol induced luteolysis. *Theriogenology* 39:1321-1330.

Gaines J. (1989). Collecting data for nutritional analysis of subfertile dairy herds. *Vet. Med.* 5:915-923.

Kamomae H. (1990). Therapeutic and physiopathological study on ovarian quiescence in cattle. *Jpn J. Anim. Reprod.* 36:11-22.

Mapletoft RJ dan Pierson RA (1993). Factors affecting superovulation in the cow: practical considerations. *Embryo Transfer Newsletter* 11:15-24.

Munro C, dan Stabenfeldt G. (1989). Development of microtitre plate enzyme immunoassay for the determination of progesterone. *J. Endocr.* 101:41-49.

Putro PP, (1991). Ovarian follicular development in cows during and after treatment with a progesterone intravaginal device. *Australian Dairy Research Council.*

Quirk SM. dan Fortume JE. (1990) Ovarian follicular dynamics in cattle. *J. Reprod. Fert.* 77:211-219.

Rajamahendran R dan Cadler MD. (1993). Superovulatory responses in dairy cows following ovulation of the dominant follicle of the first wave. *Theriogenology* 40:99-109.

Shimohira I. (1991). Twinning and in vitro fertilization technology for cattle. Japan International Cooperation Agency.

Suzuki T. (1991). *Embryo Transfer Technology in Cattle.* Yamaguchi University, Yamaguchi, Japan.

Suzuki T. (1993). Cattle embryo transfer and related techniques. *Molecular Reproduction and development.* 36:236-237.

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